Imaging with [11C]-metformin in tumor bearing mice showed a large uptake in the kidneys and excretion through the bladder, as expected for metformin. An uptake of [11C]-metformin was seen in both A549 (lung) and SiHa (cervix) tumors and autoradiography supported this finding. Biodistribution of metformin in humans is shown in figure 1 with visible uptake in liver, kidney and the salivary glands, but no detectable uptake in brain, muscle or adipose tissue.

Conclusion: It is possible to visualize distribution of [11C]-metformin *in vivo*. In xenograft models uptake in tumor was seen. It will be of great interest to investigate whether it is possible to visualize an uptake in human tumors, which will be done in a planned study in prostate cancer patients.

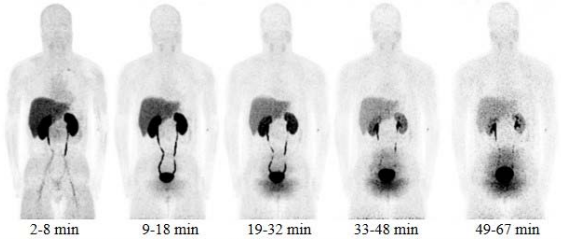

Figure 1: Tissue distribution of [^{11}C]-metformin. Healthy subjects given 200 MBq [^{11}C]-metformin i.v. Time denotes min after injection.

Poster: Radiobiology track: Cellular radiation response

PO-0995

Osteopontin expression in glioblastoma – a promoter of the cancer stem cell-like phenotype?

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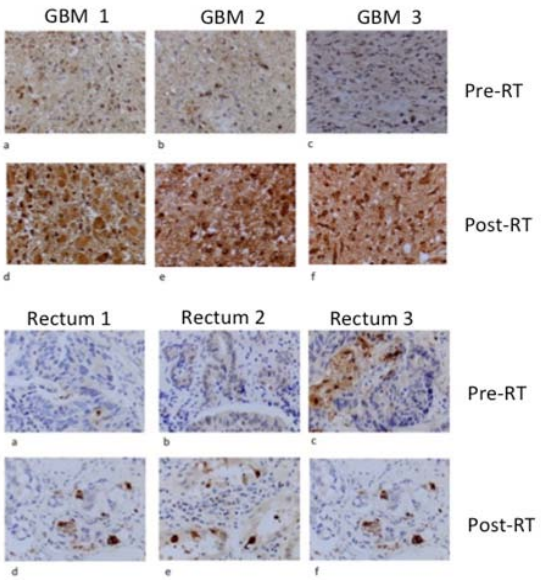
Purpose or Objective: A high level of circulating osteopontin (OPN) at the end of radiotherapy (RT) is an adverse prognostic factor in patients with glioblastoma (GBM) and other tumours including rectum cancer. Recent mechanistic studies demonstrated HIF2α-mediated OPN/CD44 promotion of the glioma stem cell-like phenotype in a mouse model. Using unique paired tumour samples from patients with GBM, we investigated changes in levels of OPN protein expression following RT and compared these with rectum cancers from patients irradiated with the same pre-operative fractionation.

Material and Methods: 3 patients with histologically confirmed GBM received pre-operative RT in an ethics-approved Phase I trial. 2.5 Gy b.d. was delivered using IMRT over 5 days. Maximal safe tumour resection was performed at 3, 5 and 10 days post RT in patients 1, 2 and 3 respectively. Immunohistochemistry was performed on the paired diagnostic biopsy and irradiated resection specimen using validated antibodies (rabbit polyclonal antibody to OPN: clone PA1-38332, Thermo Fisher Scientific) and an automated immunostainer. The staining was scored by a board-certified pathologist.

Results: Levels of OPN in GBM tumour cells were high at baseline as compared with rectum adenocarcinoma. There was marked increase in OPN expression in response to RT in all three GBM tumours (Fig 1). Expression of Glut-1, a marker of intrinsic hypoxia and a target of HIF-2α, was not induced. Ki67 levels were reduced although levels of cyclin D1 expression were unchanged. A dynamic contrast-enhanced (DCE) MRI performed on the last day of RT did not detect any change in tumour perfusion in any of the GBMs. Resection specimens from 3 rectum cancer patients irradiated preoperatively with the same schedule showed very low level induction of OPN.



Conclusion: RT increased the levels of OPN expression in GBM tumour cells. This may be a direct effect or related to RT-induced changes in the hypoxic tumour microenvironment that were not detectable on a DCE-MRI or by Glut-1 expression. Although RT significantly increases overall survival compared with surgery alone, particularly when combined with temozolomide, it may promote the cancer stem cell-like phenotype of residual GBM cells. Enhanced OPN/CD44 signalling in the perivascular niche is associated with resistance to therapy and blockade of this signalling pathway may prove of clinical benefit. The relative lack of induction of OPN expression in rectum cancer may explain the success of short course pre-operative RT in this tumour type.

PO-0996

Distinct radiation responses after mtDNA depletion are potentially related to oxidative stress

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Purpose or Objective: In process like reactive oxygen production and apoptosis mitochondria play an important role and both processes play also a significant role in radiotherapy (RT) response. Repair of RT induced damage is dependent on mitochondrial energy supply suggesting a role for mitochondrial DNA (mtDNA) in RT. mtDNA variations, such as mutations or depletion, might therefore influence RT response, as for example found in cisplatin-treated patients. Therefore carefully elucidating the effect of these processes in radiation response might be important. Hence, we hypothesize that reduced mitochondrial function enhances the radiation response as a consequence of reduced ATP production and increased cellular ROS exposure (Fig.1).

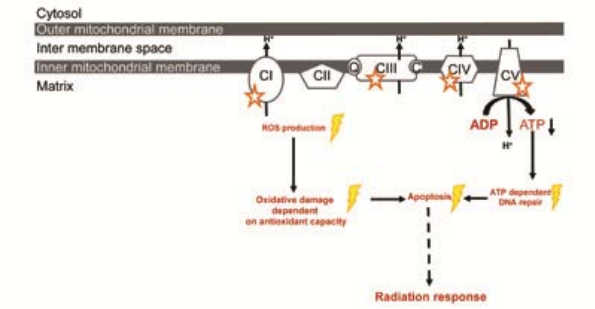

Fig 1. We hypothesize that reduced mitochondrial function caused by mtDNA variations, such as mtDNA depletion, enhances the radiation response as a consequence of reduced ATP production and increased cellular ROS exposure. Oxidative phosphorylation complexes that are encoded by the mtDNA are indicated by the orange stars. Through disruption of these complexes and thereby interfering with free radical and ATP production, radiation responses could potentially be altered. Lightning signs indicate processes that are also involved in radiotherapy.

Material and Methods: Three cell lines were depleted from their mtDNA by ethidium bromide. BEAS-2B immortalized bronchial epithelial, A549 lung adenocarcinoma and 143B osteosarcoma cell lines and their mtDNA depleted counterparts (ρ0) were metabolically characterized using the XF96 Seahorse. Changes in radiosensitivity were assessed by clonogenic survival (0, 2, 4, 6 and 8Gy). ROS production (by dihydrorhodamine FACS analysis), ATP (Cell-TiterGlo Luminescent cell viability test) and glutathione levels (in cell lysate) as well as γH2AX immunostainings were assessed 24 hours post irradiation.

Results: mtDNA depletion resulted in a significant ($p<0.05$) decreased proliferation (64 ± 7%) for all cell lines. Compared to their respective controls, increased clonogenic survival was observed for the BEAS-2B ρ0 cells ($p=0.004$) after irradiation, while both tumor ρ0 lines were more radiation sensitive ($p=0.013$), mainly at higher irradiation doses. ROS formation at baseline (0Gy) was similar ($p=0.878$) for BEAS-2B parental and ρ0, while reduced for A549 and 143B ρ0 ($p=0.021$) cells, compared to their parental counterparts. 24 hours after irradiation ROS levels were significantly ($p<0.05$) increased for all parental cell lines, while levels for the ρ0 cells remained equal. Glutathione levels were lower for the A549 and 143B ρ0 cell lines compared to the parental lines under any experimental condition but no changes were found for the BEAS-2B cells. In agreement, increased residual DNA damage was observed upon mtDNA depletion for A549 and 143B cells. Depletion of mtDNA reduced cellular ATP levels only for the BEAS-2B cell line ($p=0.046$), but not for the A549 and 143B cell lines in high glucose culture medium.

Conclusion: The observed differences in dependence on mitochondrial function for radioresponsiveness appear to be associated with the balance in ROS levels and the antioxidant status of the cells. Currently, the levels of MnSOD and GPX1 and the effect of ROS scavenging on radiotherapy response are investigated in our lab.

PO-0997

Interferon response genes in breast cancer resistance to endocrine treatment and radiotherapy

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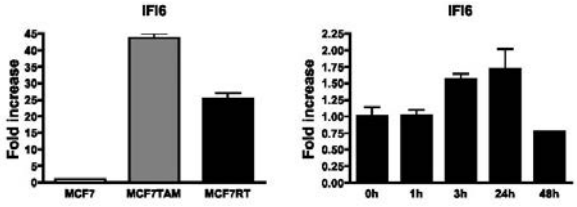
Purpose or Objective: We have previously shown that lysosome-associated membrane protein-3 (LAMP3), a protein involved in the unfolded protein response pathway, is involved in resistance to both endocrine (tamoxifen) treatment and radiotherapy in breast cancer patients. We have created subclones of the MCF7 breast cancer cell line that are resistant to either treatment. In these subclones, we investigated common mechanisms between tamoxifen- and radioresistance, and the possible role of LAMP3 therein.

Material and Methods: The estrogen receptor positive breast cancer cell line MCF7 was grown to tamoxifen resistance (MCF7TAM) by culturing with gradually increasing concentrations of 4-OH-tamoxifen up to 10 μM. Additionally, MCF7 cells were exposed to multiple fractions of 2 or 4 Gy irradiation, adding up to a total dose of at least 50 Gy (MCF7RT). Changes in expression profiles in MCF7TAM and MCF7RT cells compared to parental MCF7 cells were investigated by RNA sequencing. Pathway analysis software was used to find pathways involved in tamoxifen- and radioresistance. QPCR was used to confirm the RNA sequencing data, and to investigate the changes in genes of interest after tamoxifen treatment and irradiation.The role of LAMP3 in these treatment resistance pathways is being elucidated by performing LAMP3 gene silencing by siRNA and CRISPR-Cas mediated gene knockout.

Results: The MCF7TAM cells were completely resistant to treatment with 10 μM 4-OH-tamoxifen. Remarkably, these cells had also become resistant to irradiation, with a surviving fraction at 4 Gy (SF4) of 19.7%, compared to 8.3% for the parental MCF7 cells. MCF7RT cells were less sensitive to irradiation with a SF4 of 9.6% compared to 3.9% for the parental cells. RNA sequencing of MCF7TAM and MCF7RT cells revealed an increase of genes involved in the antiviral response, including classic interferon response genes such as IFI6 (shown in figure, left), IFI27, STAT1, OAS1 and DDX60. These genes were increased in parental cells following 4 Gy irradiation (figure, right) or tamoxifen treatment as well.



Conclusion: MCF7 cells resistant to tamoxifen treatment are also less sensitive to irradiation,suggesting a common mechanism in the resistance to these diverse types of treatment. Using an unbiased approach, we here show that interferon response genes are increased in both MCF7TAM and MCF7RT cells. Interestingly, others have shown LAMP3 to be a regulator for this pathway. We are currently investigating the role of LAMP3 in our treatment resistant breast cancer clones.

PO-0998

The Robo1-receptor is involved in the migration of irradiated glioblastoma cells

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Purpose or Objective: The brain tumor glioblastoma multiforme (GBM) is highly malignant with a very short OS due to rapid recurrences adjacent to the primary tumor. Even radio-chemotherapy extends the survival only for a few months. In this project we tested whether or not the Slit2/Robo1 axon guidance system might be involved in the migration of metastatic GBM cells and whether irradiation with photons might modify this putative effect.

Material and Methods: The experiments were performed with 2 human GBM cell lines (U87 and U373) and in parallel after irradiation with 0.5, 2, or 8 Gy photons. The motility/migration of the cells was analyzed by time-laps videography. Travelling cells were tracked and the parameters accumulated distance and Euclidean distance were determined. The expression of Slit2, Robo1, and FAK (focal adhesion kinase) was tested by Western blot and qRT-PCR. In addition, the cells were transfected either with a Robo1 expression-vector or with a siRNA construct and analyzed similarly.